Cancer Stem Cells and Molecular Biology Test in Colorectal Cancer: Therapeutic Implications

Rustam Effendi-YS

Department of Internal Medicine, Faculty of Medicine Universitas Sumatera Utara - Adam Malik Hospital - Pirngadi Hospital, Medan, Indonesia.

Corresponding author:
Rustam Effendi-YS. Division of Gastroenterology Hepatology, Department of Internal Medicine, Faculty of Medicine Universitas Sumatera Utara - Adam Malik Hospital - Pirngadi Hospital. Jalan Sutrisno/Sehati 754 Medan 20216, Indonesia. email: effendiysr@yahoo.com, rustam_e_ys@yahoo.com.

ABSTRAK

Kanker kolorektal (KKR) merupakan kanker urutan ketiga terbanyak pada laki-laki dan kedua pada perempuan, dan penyebab kematian ke dua akibat kanker di dunia. Indonesia dengan penduduk sekitar 250 juta dan incidence rate *KKR per 100.000 populasi 15,2 untuk laki-laki dan 10,2 perempuan, diperkirakan memiliki 63.500 kasus per tahun. Lebih dari 50% penderita KKR akan mengalami metastasis. KKR masih merupakan penyebab utama kematian akibat kanker, dan dari sejumlah pasien yang telah menjalani operasi KKR, sebagian akan kambuh kembali. Kemoterapi juga mempunyai beberapa tantangan termasuk adanya resistensi terhadap kemoterapi. Pemeriksaan molekuler jaringan tumor menjadi penting dan mempunyai implikasi dalam pemilihan terapi. Pemeriksaan biomarker dapat digunakan untuk penilaian prognosis, faktor prediksi dan target terapi. Penelitian terkini menunjukkan bahwa cancer stem cells (CSCs) merupakan sumber tumorigenesis, perkembangan, metastasis dan penyebab kambuhnya kanker. Tinjauan ini mengemukakan masalah terkait CSCs dan perkembangan obat masa depan yang ditujukan untuk CSCs.*

Kata kunci: *kanker kolorektal, cancer stem cells, biomarker, kemoresisten, target terapi.*

ABSTRACT

Colorectal cancer (CRC) is the third most frequent cancer in males, the second in females, and is the second leading cause of cancer related death worldwide. Within Indonesia's 250 million population, the incidence rates for CRC per 100,000 population were 15.2 for males and 10.2 for females, and estimated 63,500 cases per year. More than 50% of colorectal cancer patients will develop metastasis. CRC is still the main cause of tumor-related death, and although most CRC patients are treated with surgery to remove the tumor tissue, some of the CRC patients recurred. Chemotherapy used as adjuvant or neoadjuvant therapy also has several problems, in which these treatments are useless in tumor cells with chemo-resistance. Molecular testing of CRC from tumor tissues has important implications for the selection of treatment. Biomarkers can be used as prognostic value, molecular predictive factors, and targeted therapy. Recent research reported that, cancer stem cells (CSCs) are considered as the origin of tumorigenesis, development, metastasis and recurrence. At present, it has been shown that CSCs existed in many tumors including CRC. This review aims to summarize the issue on CSCs, and the future development of drugs that target colorectal cancer stem cells.

Keywords: *colorectal cancer, cancer stem cells, biomarkers, chemoresistance, target therapy.*

INTRODUCTION

Colorectal cancer (CRC) is the third most most frequent cancer in males, the second in females, and the second leading cause of cancer death worldwide, with estimated 1.4 milion new cases and 693,900 deaths in 2012. Indonesia, with its 250 million population, has age-standardized incidence rates for colorectal cancer per 100,000 population were 15.2 for males and 10.2 for females, and has estimated 63,500 cases per year, and an almost similar burden to other countries with increasing populations.[1-3]

Despite advanced treatment strategies, the disease is rarely cured completely due to recurrence. Evidence shows that this is due to a small population of cells, called cancer stem cells (CSCs), in the tumor mass that have the self-renewal and differentiation potential to give rise to a new tumor population. Tumor recurrence remains a major challenge in the treatment of CRC, due to the presence of the CSC population which contributes to chemo-resistance.[4]

Sporadic CRC derives from somatic mutations, and is not associated with family history. CRC is a heterogeneous complex of diseases. Progression to CRC is considered a multi-step process, with an accumulation of various genetic and epigenetic alterations, leading to transformation from a normal cell to a premalignant tumor and finally to malignant and potentially metastatic tumor.[5-8]

Clinical applications of the molecular biological marker status of CRC patiens are the important factors to determine either the possibility of successful treatment or response of chemotherapy (i.e. predictive factor) or life expectancy (i.e. prognostic factor). Cancer locations, whether the tumor is at proximal, distal colon or rectum, are also different in their associated molecular alterations in carcinogenesis, and also in the treatment response with chemotherapy.[9-12] Approved target therapy for CRC consists of the anti epidermal growth factor receptor (anti-EGFR) cetuximab, and anti vascular endothelial growth factor receptor (anti-VEGF monoclonal antibody) bevacizumab. KRAS mutations predict lack of response to therapy with anti-EGFR. CRC with MSI-high status is said to be resistant to 5-FU.[13-16]

Recent research reported that cancer stem cells (CSCs) are considered as the origin of tumor genesis, development, metastasis and recurence in theory. At present, it has been proved that, CSCs exist in many tumors including CRC.[17-18]

MOLECULAR BIOLOGY TESTS

Treatment approache by using the molecular test results of CRC tumor tissue will give information for the selection of individualized therapy, representing the principles of personalized tumor diagnostics and targeted therapy. Fresh tissue is obtained from colorectal tumors, either by colonoscopic biopsy or surgery. Tumor tissues are immediately fixated by using 10% formaldehyde buffering solution and made into paraffin blocks or formalin-fixed and paraffin-embedded tissues (FFPET. It is used for the examination of histology of the tumor, and immunohistochemical test (IHC) for the evaluation of the protein expression negative (PEN) of chromosomal instability (CIN), and DNA-mismatch repair protein (MMR) defect or the assessment of microsatellite instability (MSI) status. Tumor tissue is also used for examining MSI by polymerase chain reaction (PCR), and CpG island methylation phenotype (CIMP) status.[11,19,20,21]

Chromosomal Instability (CIN)

It is suggested that CIN is the most common (80-85%) genomic instability of all CRC and adenomas. CIN induces carcinoma through the loss or mutation of tumor suppressor genes, such as APC, TP53, and also through activation of oncogens such as KRAS. CRC caused by CIN usually have poor prognosis. KRAS gene plays the most important role. The activation of RAS genes can promote cell survival and suppress apoptosis. Most KRAS mutations occur in codon 12 (70-80%), and codon 13 of exon 2. KRAS mutation analysis is widely used as a prognostic and predictive biomarker in treating CRC patients, to predict the therapeutic effectiveness of anti-EGFR monoclonal antibody (such as cetuximab and panitumumab). KRAS mutations predict lack of response to those anti-EGFR. Mutations in BRAF occur in approximately 12% of all CRCs patients and it is mutually exclusive of KRAS mutation. Investigation

of BRAF mutations is also recomended when KRAS mutation are not found. Adenomaous polyposis coli (APC) gene plays a crucial role in the Wnt/Wingless pathway. APC gene is the most important gatekeeper of colonic epithelial cell proliferation and it is responsible for controlling the underlying oncoprotein called β-catenin. The loss of function in APC gene may lead to the transition to adenoma from normal colonic mucosa due to the up-regulation of β-catenin. Somatic APC mutations are present in most sporadic colorectal adenomas and cancers. Similar to KRAS, APC mutations appear in the early stage of the progression from adenoma to carcinoma.[6,20,21]

Deoxyribonucleic Acid (DNA) Mismatch Repair (DNA-MMR) and Microsatellite Instability (MSI)

Approximately 15% of all CRCs show underlying defects in DNA-MMR (dMMR) and the tumor tissues show MSI positive or MSI high (MSI-H). Furthermore, 3-5% MSI-H of CRC patients harbor germline mutations related to the Lynch syndrome and 12% are sporadic colorectal cancer.[22] MSI-H status is correlated with the tumors being in the proximal colon and improved survival. At least six different genes (e.g. MSH2, MLH1, PMS1, hPMS2, MSH6, and MLH3) encode the MMR system. CRC patients with MSI-H status receiving fluorouracil (5-FU) showed no improvement in disease-free survival, and infact it was associated with reduced overall survival.[11,19,21]

The underlying cause of MSI-H can be explained by two mechanism: 1) defective MMR system, in which both allels of a MMR gene (e.g. MLH1, MSH2, MSH6, and PMS2) are non-functional. This results in the loss of ability to repair DNA replication mismatches in the affected cells; 2) hypermethylation of promoter in MMR genes that suppress the expression of the genes like MLH1.[6,20,23]

In our sudy, there were the differences in the characteristics of CIN, MMR, and MSI-H found in colorectal cancer patients based on different locations. The protein expression negative were examined by IHC for APC, MMR (MLH1, MSH2, MSH6 and PMS2), and for MSI-H by PCR based on 5 markers of BAT25, BAT26, D2S123, D5S346, D17S250. MSI-H was considered if there were ≥ 2 of abnormal markers.[11,12]

The MSI classification system is highly valuable in prognosis and therapy since chemotherapy using 5-fluorouracil is not efffective in treating MSI-H tumors. Instead irinotecan-containing regimens have shown improved responses and better prognosis for MSI-H tumors.[23,24]

Epigenetic Gene Silencing

Cancers with high degree of DNA-methylation can be considered as CpG island methylation phenotype (CIMP) positive, and it encompasses 35-40% of sporadic CRC. CpG hypermethylation can lead to silencing of tumor-suppressor genes in carcinogenesis since the expression of the genes is repressed. In some cases, the presence of epigenetic silencing overlapps with MSI-H. Some sporadic CRC with MSI-H is caused by DNA methylation.[6,20,25]

MicroRNAs (miRNAs)

MicroRNAs are small non-coding RNA that are usually 19-23 nucleotids in length. Due to their small size, miRNAs are more stable in blood and FFPE tissues than other nucleic acids such as DNA and RNA. miRNAs are involved in post-transcriptional regulation of gene expression. Therefore, they are able to function as oncogens or tumor suppressor genes, and dysregulation of miRNAs would be associated with cancers. The miR-135a and miR-135b play important roles in the regulation of the Wnt/Wingless pathway by down-regulating APC gene expression. miR-17-3p and miR-92a have been found to be elevated in plasma and their levels decreased after removal of the cancer tissues. These results suggested that circulating miR-92 and miR-17 are potential non-invasive diagnostic markers for CRC. Apart from the miRNAs mentioned above, miR-211 is also believed to be a potential marker for the diagnosis and prognosis of CRC.[21,26,27]

Cancer Stem Cells (CSCs)

Most cancers are heterogeneous and show functional and phenotypical differences at the cell population level. Evidence continues to accumulate showing that tumors containing a minority population of cells responsible for

tumor initiation, growth, and recurrence. These are termed "cancer stem cells" (CSCs).[28] CSCs possess both the self-renewal and differentiation capabilities or tumor-initiation capabilities of CSCs. Moreover, recent studies have revealed that these CSCs is responsible for chemotherapy resistance within a tumor. Several mechanisms of chemoresistance have been proposed, including several signaling pathways that are involved in the self renewal behavior of CSCs, including Wnt/β-catenin and Notch, and Hedgehog signaling, which mediate the resistances against radiotherapy and chemotherapy.[29,30]

Stem cells of the colon crypt are the origin of colon mature cells. CRC cells are also suggested to originate from crypt stem cells undergoing a series of epigenetic and genetic alterations. Aberrant methylation plays an important role in early carcinogenesis and lead to altered gene expression and regulation, resulting in accumulation of damages to cell function and ultimately, malignant transformation. Aberrances in hypermethylation and hypomethylation act as different mechanism through the regulation of various genes during CSC carcinogenesis, and both of them play crucial roles in stem cell differentiation toward cancer cells. A large majority of epigenetic and genetic abnormalities that work coordinately in colorectal carcinogenesis are related to cell growth and division, indicating that the intrinsic abnormalities of CRC lie in dysregulation of basic cellular process. Detection of abnormal methylation can be used in cancer screening and early detection, while reversal of aberrant methylation using drugs may have potential in cancer therapy.[32]

Epigenetic and genetic abnormalities are definite risk factors for CRC, as DNA methylation and gene mutations are both found in colon precancerous and cancerous tissues. Epigenetic mechanisms, including DNA methylation, are actively involved in the regulation of normal intestinal stem and progenitor epithelial cells through the repression or activation of differentiation or proliferation genes.[32-33]

The concept of cancer stem cell (CSC) was proposed as studies found epigenetic and genetic aberrances in progenitor or precursor cells that have the capacity to differentiate into certain cell types.[33] Research showed that the malignant transformation of cancer starts at very early stage when the cells are still at the stem cell stage.

Two models are currently available for explaining the process of CRC carcinogenesis:

a) In the CRC somatic cell model, cancer cells are suggested to originate from normal mature epithelial cells. These cells undergo a series of epigenetic alterations, such as MLH1 gene methylation, and genetic alterations, such as MSI pathway initiated by MMR gene mutation. Aberrant methylation and mutations are associated with CIN and further mutations in key regulatory genes, such as KRAS, BRAF, and lead to progression from low-grade abnormal proliferation (e.g. small adenoma) to high-grade abnormal proliferation (e.g. large adenoma or advanced adenoma). The accumulation of aberrant methylation and further mutations (TP53,TGFBR2 and BAX, etc) leads to malignant transformation of epithelial cells. Abnormal methylation and key gene mutations are regarded as the initial changes for epithelial cell transformation to ward cancer cells.[6]

b) The CSC model suggest that tumors are hierarchially organized and only CSCs posses cancer promoting properties. In the CRC cancer stem cell model, malignant changes are suggested to start at the stem or precursor cell level. Precursor cells are a type of partially differentiated stem cell which has the capacity to differentiate into one cell type, and therefore are also called unipotent stem cells. Epigenetic changes, such as aberrant methylation, may result in silencing of genes p16, SFRPs, GATA-4/-5 and APC in stem/precursor cells of adult cell renewal systems and may lock these cells into stem-like states that foster abnormal cell clonal expansion, and the stem/precursor cells are transformed into preinvasive cancer stem cells.[34] The silencing of key genes can foster increased CRC-related pathway signaling (such as Wnt pathway), resulting in genomic instability and mutations in the downstream pathway genes, such as APC or β-catenin, and further activate these signaling pathways to foster colon tumorigenesis.[35,36] At this stage, preinvasive cancer stem cells turn into cancer stem cells that will ultimately

become cancer cells due to accumulation of epigenetic and genetic abnormalities. It is clear from the models that both epigenetic and genetic alterations play crucial roles in CRC carcinogenesis, while epigenetic alterations may be a predominant factor during early malignant transformation of colonic stem cell in the stem cell model.

The proposal of the CRC stem cell model has great significance in guiding CRC therapy. The current chemotherapies generally aim at mature cancer cells, not the CRC CSCs. Although these treatments can reduce the size of cancer tissue, they cannot completely kill CSCs that have higher proliferative potential and stronger resistance to chemotherapy than mature cancer cells. They can escape the chemotheapy and differentiate into mature cancer cells when therapy is withdrawn, resulting in cancer recurrence and metastasis. Therefore, development of therapy targeting CSCs has a therapeutic potential to achieve better treatment to suppress cancer growth and metastasis.[32]

Markers of Colorectal Cancer Stem Cells

Several stem cell markers, including CD133, CD44, CD24, CD166, LGR-5 and aldehyde dehydrogenase-1 (ALDH-1) are currently being developed as CR-CSCs markers (**Tabel 1**). Drug resistance exists in CR-CSCs may be associated with the slow growth of CR-CSCs in G0 stage. A large sample of clinical research conducted in 501 CRC patients demonstrated that, tumors with CD133 overexpression showed more resistance in 5-FU based chemotherapy and the expression CD133 related to poor prognosis.[37] MicroRNAs (miRNAs) also play a role in the regulation of cell proliferation and can regulate CR-CSCs specific signal pathway to increase CR-CSCs drug resistance. For example, miRNA-140 inhibits the

Table 1. The markers of colorectal cancer stem cells.[17]

Marker	Function	Significance
CD133 (prominin-1)	Pentaspan transmembrane glycoprotein, organization of plasma membrane	Tumor initiation in xenografts, colony formation, correlation with: Poor prognosis, ↓survival, recurrence, metastasis, resistance to therapy
CD44	Cell adhesion molecule, hyaluronic acid receptor	Tumor initiation in xenografts, colony formation, association with tumor stage, lymph node infiltration, prognosis and survival
CD166 (ALCAM)	Cell adhesion molecule	Tumor initiation in xenografts, colony formation, further enrichment, correlation with prognosis and survival
CD24	Cell adhesion molecule	↑Clonogenic ability, multilineage potential, further enrichment, correlation with invasiveness, differentiation and survival
ALDH-1	Detoxifying enzyme, resistance to alkylating agents	Tumor initiation in xenografts, further enrichment, transition from colitis to cancer, mitochondrial isoform is highly↑in CRC
CD29 (β1-integrin)	Cell adhesion molecule	Colony formation, in CRC↑, association with tumor stage, ↑mRNA in CRC
Msi-1	RNA-binding protein, maintenance of the undifferentiated state of SCs	Expression in CD133+ cells and spheroid cultures, ↑in CRC, association with tumor stage, ↑mRNA in CRC
Wnt pathway activity, b-catenin	Maintenance and proliferation of the SC reservoir	↑Activity is associated with: Clonogenicity and tumorigenicity, detection of low stage CRC cases with: Risk of relapse
LGR-5	Wnt target gene, crypt base restriction, unknown function	↑Tumorigenicity, poor prognostic factor, metastasis formation, in a APC knockout mouse model LGR-5+ cells form adenomas within weeks
EpCAM (ESA)	Cell adhesion molecule	Expression in CD133+ or CD44+ cells
CD26	Cell surface glycoprotein	Tumor initiation and metastasis formation in a mouse model
Oct-4, Sox-2, Nanog, Lin-28, Klf-4, c-myc	Transcription factors	Correlation with poor prognosis, relapse, distant recurrence, resistance to therapy

proliferation of CD133+high/CD44+high human CRC cells through regulating histone deacetylase 4, leading to resistance to 5-FU.[38]

The exact relationship between CR-CSCs and chemotherapy is still unclear, several studies demonstrated that in CRC, CD133+ CR-CSCs survived after adjuvant chemotherapy. As the treatment dose increased, the expression of CD133 also increased in vitro.[39,40]

COLORECTAL CSCS AND THERAPEUTIC IMPLICATIONS

Currently, most of the interventional clinical trials are carried out on metastatic patients with the aim of temporarily controling tumor progression. However, with the increasing knowledge of colorectal CSC (CR-CSC) biology, several therapeutic options are becoming available. The effectiveness of CSC-targeted therapies may be maximal on CSCs disseminated outside the tumor context, which may be vulnerable to combined therapies due to the absence of a protective niche. In fact, a partial targeting of disseminated CSCs may be empirically carried out in the clinic during adjuvant treatment. Although there is no formal proof that CSCs can be effectively targeted in CRC patients, it is extremely likely that adjuvant chemotherapy can kill disseminated CSCs that escape surgery. This would explain why there is a statistically significant curative advantage in the administration of adjuvant therapy in stage II and III patients.[41] New targeted therapy strategies for advanced colon cancer are provided in **Table 2**. The purpose of this therapy is to control the relevant pathways of tumor growth, survival and metastasis.[13,14]

Colorectal Cancer Targeted Therapy

Current therapies do not target the CSC. This could be the reason behind recurrence of tumor growth or resistance to treatment post therapy.[39,40] Many studies have investigated different ways to target tumorigenesis. One focus of recent research has been the usage of markers. Markers are molecules on the cell surfaces of these mutated cells. These markers are used in attempts to identify potential target of therapy. Another form of targeting cancer growth is via pathways. The regulation of the stem cells survival is through the signaling pathway such as Wnt pathway. Markers for the mentioned pathways may enable us to locate stem cells and monitor progression. CRC can be treated in the early stages via surgery, however if not diagnosed and treated in time, the rate of survival diminishes rapidly. All CRC chemotherapy treatments target the proliferative nature of the cancer cells in an attempt to minimize the growth. It seems as if the stem cells are not being targeted or perhaps provide resistance to the therapies themselves. The CSC may be capable of regenerating cells resistant to the therapy. The future of treatment of CRCs lies in the new and upcoming research on CSCs. The potential in CSC targeting via markers is vast in account of

Table 2. Novel targeting approaches against colorectal cancer stem cells[17]

Gene/pathway	Targeting mechanism	Targeting mechanism
VEGF	Bevacizumab, humanized anti-VEGF mAb	FDA approved
EGFR	Cetuximab, an mAb against the EGFR extracellular binding domain	FDA approved
IGF-IR	IGF-IR inhibitor, AVE-1642	Experimental
IL-4	Anti-IL-4 neutralizing antibody	Experimental
Aurora-A kinase	Specific gene silencing	Experimental
Tcf/(3-catenin complex)	Small molecule inhibition	Experimental
Notch pathway	γ-secretase inhibitor	Experimental
BMP4 pathway	Recombinant BMP4	Experimental
Thymosin β4	Specific gene silencing	Experimental

VEGF=Vascular endothelial growth factor, EGFR=Epidermal growth factor receptor, IGF-IR=Insulin-like growth factor-I receptor, IL-4=Interleukin-4, BMP=Bone morphogenesis protein, FDA=Food and Drug Administration

the benefits regarding treatment. If these CSCs can be found and pathways better understood, chemoradiotherapy (CRT) resistance cancers can be targeted, offering a better prognosis than the current situation.[42]

Traditional chemotherapy should be combined with new strategies targeting CSCs to prevent tumor relapse and to provide a highly-efficient and low toxic treatment for cancer therapy.[43]

Targeting the Molecular Signaling Pathways

Signaling pathways are essential for normal stem cells related to self-renewal, proliferation and differentiation; however, the dysregulation or aberrant activation of these key pathways may result in the formation of cancer stem cells (CSCs) which induce tumorigenesis. These important signaling pathways consist of Hedgehog (Hh), Notch,Wnt/β-catenin signaling pathway, high mobility group AT-hook 2 (HMGA2), Bcl-2, Bmi-1, and more. The most studied and characterized pathways are Hh, Notch, Wnt/β-catenin signaling pathway, which are responsible for the formation of CSCs. Therefore, targeting these aberrant signaling pathways are important offers a new strategy for cancer therapy.[43]

The Wnt/β-catenin signaling pathway has a role in maintaining the phenotype of intestinal crypt stem cell. APC function loss or β-catenin mutation induce Wnt signaling disorder are the leading causes of most CRC. LGR5, a marker of intestinal stem cell could regulate CRC cell proliferation and survival through targetting the Wnt/β-catenin signaling pathway. CD44v6 is found to be expressed in all CR-CSCs, inhibition of phosphatidylinositol 3-kinase selectivity killed CD44v6 CR-CSCs and decreased metastatic growth.[17,44-48]

The Notch signaling pathway is overexpressed in CR-CSCs, and plays a role in CR-CSCs tumorigenecity and self-renewal by inhibiting cell-cycle kinase inhibitor p27 and transcription factor ATOH1. In APCmin CRC model, γ-secretase inhibition mediated Notch signaling dysfunction induced the APC min proliferating cells in intestinal adenoma turning into goblet cells, then leading to the growth stagnation of tumor cells. BMP4 plays a key role in the development of physiological intestinal crypts. Mutation of BMP4 is signaling pathway (SMAD4 and BMPRIA) or blocking the BMP4 signaling pathway by expression of BMP4 antistatic agent. Noggin induces the development of intestinal crypts, cancer susceptibility syndrome, and juvenile polyposis syndrome. The expression of BMPR2 and SMAD4 were also found in an immunohistochemical analysis of 72 sporadic CRC patients, revealing that blocking BMP4 signaling pathway can potentially contribute o the formation of colorectal tumor. In this aspect BMP4 could induce the differentiation of CD133 + CR-CSCs, improving the sensitivity of oxaliplatin and 5-FU in the treatment of xenografted tumor. It is reported that, actin-binding peptide thymosin β4 (T β4) is overexpressed in CD133 + CR-CSCs. Targeting T β4 can damage the growth and migration of CR-CSCs in vitro, also through regulating integrin-linked kinase/Akt transduction cascades invivo can reduce the tunor size of CD133 + CR-CSCs based xenografts in mice.[17,49-53]

Targeting CSCs Markers

Markers used to isolate, identify and enrich CSCs are also ideal targets for cancer therapy. Targeting cytotoxic drugs to CSCs with the help of stem cell surface markers provides a useful method to treat cancer. Also, the use of inhibitors targeting drug-detoxifying enzymes, drug-efflux pumps, or transcription factors of CSCs represents a novel approach to target the CSCs and reduce cancer recurrence and metastasis.[43]

CONCLUSION

Chemotherapy can only shrink tumors by killing the active tumor cells but miss the quiescent CSCs that lead to resistance and relapse, and may even enrich CSCs for a more resistant state, and usually include systemic and local toxicity. New treatments targeting CSCs are necessary for improving patient survival rate and prolonging life span. Thus, more precisely targeted therapies which can selectively target CSCs but spare normal stem cells are greatly needed. Molecular markers have therapeutic implications for individualized treatment in

colorectal cancer patients. Several biomarkers associated with predictive and prognostic values are available. The markers of colorecal cancer and targeting approaches against colorectal cancer stem cells are being developed.

REFERENCES

1. Torre LA, Bray F, Siegel RL, Ferlay J, Tieulent JL, Jemal A. Global cancer statistics. Ca Cancer J Clin. 2015;65:87-108.
2. GLOBOCAN 2012. Estimated cancer incidence, mortality and prevalence worldwide in 2012 Icited:March 9,2016). Available from url:http://globocan.iarc.fr/Dafaul.aspx.
3. Effendi-YSR. Economic burden of colorectal cancer: Prevention vs Treatment. In: Simadibrata M, Makmun D, Abdullah M, Syam AF, eds. Proceeding Book. Indonesian Digestive Disease Week. Jakarta: Interna Publishing; 2016. p. 50-8.
4. Ramasamy TS, Ayob AZ, Myint HHL, Thiagarajah S, Amini F. Targeting colorectal cancer stem cells using curcumin and curcumin analogues: insights into the mechanism of the therapeutic efficacy. Cancer cell Int.2015;15:96 http://doi.org/10.1186/s12935-015-0241.
5. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;61:759-67.
6. Markowitz SD, Bertagnolli MM. Molecular orgins of cancer: Moecular basis of colorectal cancer. N Engl J Med. 2009;361:2449-60.
7. Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC,Dirks PB. Tumour-initiating cells: Challenges and opportunities for anticancer drug discovery. Nat Rev Drug Discov. 2009;8:806-23.
8. Collusi D, Brandi G, Bazzoli F, Ricciardiello. Molecular pathways involved in colorectal cancer: Impications for disease behaviour and prevention. Int J Mol Sci. 2013;14:1635-85.
9. Zoratto F, Rossi L, Verrico M, et al. Focus on genetic and epigenetic events of colorectal cancer pathogenesis: Implications for molecular diagnosis. Tumour Biol. 2014;35:6195-206.
10. Farkas SA, Vymetalkova V, Vodickova L, Vodicka V, Nilsson TK. DNA methylation changes in genes frequently mutated in sporadic colorectal cancer and in the DNA repair and Wnt/β-catenin signaling pathway genes. Epigenomics. 2014;6:179-91.
11. Effendi YSR, Zain LH, Siregar GA, et al. Adeno atous polyposis coli, mismatch repair, and microsatellite instability in colorectal cancer based on locations. Acta Med Indones. 2013;45(4):275-83. PubMed.http://www.ncbi.nlm.nih.gov/pubmed/24448331#.
12. Effendi YSR, Rey I. Molecular diagnostics in colorectal cancer. Indones J Gastronterol Hepatol Dig Endosc. 2015;16(1):26-33.
13. Seal BS, Sullivan SD, Ramsey SD, et al. Systemic therapy for colorectal cancer: Patterns of chemotherapy and biologic therapy use in in nationally representative US claims database. Bio Drugs. 2014;28:229-36.
14. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. N Engl J Med. 2005;352:476-87.
15. Chen X, Liu Y, Yang HW, et al. SKLB-287.a? novel oral multikinase inhibitor of EGFR and VEGFR2, exhibits potent antintumor activity in LoVo colorectal tumor model. Neoplasma. 2014;61:514-22.
16. Feng QY, Wei Y, Chen JW, et al. Anti-EGFR and anti-VEGF agents: Important targeted therapies of colorectal liver metastases. World J Gastroenterol. 2014;20:4263-75.
17. Jie Li C, Zhang X, Wei Fan-G. Updates in colorectal cancer stem research. J Can Res Ther. 2014;10:233-9.
18. Frank NY, Schatton T, Frank MH. The therapeutic promise of the cancer stem cells concept. J Clin Invest. 2010;120:41-50.
19. Stone JG, Robertson D, Houlston RS. Immunohistochemistry for MSH2 and MLH1: a method for identifying mismatch repair deficient colorectal cancer. J Clin Pathol. 2001;54:484-7.
20. Legolvan MP, Taliano RJ, Resnick MB. Application of molecular techniques in the diagnosis, prognosis and management of patients with colorectal cancer: a practical approach. Hum Pathol. 2012;43:1157-68.
21. Huth L, Jakel J, Dahl E. Molecular diagnostic applications in colorectal cancer. Microarrays. 2015;3:168-79.
22. Sohal D, Sepulveda AR, Sun W. Targeted therapies and molecular diagnostics of gastrointestinal cancer. In: Sepulveda AR, Lynch JP, eds. Molecular pathology of neoplastic gastrointestinal diseases. New York: Molecular Pathology Library. Springer Sci +Business Media. 2013. p. 33-41.
23. Vilar E, Gruber SB: Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol. 2010;7:153-62.
24. Al-Sohaily S, Biankin A, Leong R, Kohonen-Corish M, Warusavitame J. Molecular pathways in colorectal cancer. J Gastroenterol Hepatol. 2012;27:1423-31.
25. Kanwal R, Gupta S. Epigenetic modifications in cancer. Clin Genet. 2012;81:303-11.
26. Tsang AH, Cheng KH, Wong ASP, et al. Current and future molecular diagnostics in colorectal cancer and colorectal adenoma. World J Gastroenterol. 2014;20:3847-57.
27. Hrasovec S, and Glavac D. MicroRNA as novel biomarkers in colorectal cancer. Front Genet. 2012;3:1-9.
28. Wang Ke, Wu X, Wang J, Huang J. Cancer stem cell theory: therapeutic implications for nanomedicine. Int J Nanomedicine. 2013;8:899-908.
29. Gangemi R, Paleari L, Orengo AM, et al. Cancer stem cells: a new paradigm for undersanding tumor growth and progression and drug resistance. Curr Med Chem. 2009;16(14):1688-1703.

30. McCubrey JA, Abrams SL, Stadelman K, et al. Targeting signal transduction pathways to eliminate chemotherapeutic drug resistance and cancer stem cells. Adv Enzyme Regul. 2010;50(1):285-307.
31. Rich JN, Bao S. Chemotherapy and cancer stem cells. Cell Stem Cell. 2007;1(4):353-5.
32. Song L, Li Y. The role of stem cell DNA methylation in colorectal carcinogenesis. Stem Cell Rev and Rep DOI 10.1007/s12015-016-9672-6. Springer. Published online: 11 July 2016.
33. Roostaee A, Benoit YD, Boudjadi S, Beaulieu JF. Epigenetics in intestinal epithelial cell renewal. J Cell Physiol. 2016;9999:1-7.
34. Jones PA, Baylin SB. The epigenomics of cancer. Cell. 2007;128:683-92.
35. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer- a mechanism for early oncogenic pathway addition? Nature Rev Cancer. 2006;6:107-16.
36. Song L, Li Y, He B, Gong Y. Development of small molecules targeting the Wnt signaling pathway in cancer stem cdells for the treatment of colorectal cancer. Clin Colorectal Cancer. 2015;14:133-45.
37. Ong CW, Kim LG, Kong HH, et al. CD133 expression predicts for non-response to chemotherapy in colorectal cancer. Mod Pathol. 2010;23:450-7.
38. Song B, Wang Y, Xi Y, et al. Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. Oncogene. 2009;28:4065-74.
39. Saigusa S, Tanaka K, Toiyama Y, et al. Clinical significance of CD133 and hypoxia inducible factor-1a gene expression in rectal cancer after preoperative chemoradiotherapy. Clin Oncol (R Coll Radiol). 2011;23:323-32.
40. Saigusa S, Tanaka K, Toiyama Y, et al. Immunochemical features of CD133 expression: Association with resistance to chemoradiotherapy in rectal cancer. Oncol Rep. 2010;24:345-50.
41. Zeuner A, Todaro M, Stassi G, De Maria R. Colorectal cancer stem cells: From the crypt ti the clinic. Cell Stem Cell. 2014;15:692-705.
42. Ahmad A, Habeeb Z, Al-Mousa M, Al-Shawaf G, Al-Awadi M, Algooneh A. Cancer stem cells, therapeutic implications. Kuwait Med J. 2015;47(2):97-114.
43. Han L, Shi S, Gong T, Zhang Z, Sun X. Cancer stem cells: therapeutic implications and perspectives in cancer therapy. Acta Phar Sinica B. 2013;3(2): 65-75.
44. Wu WK, Wang XJ, Cheng AS, et al. Dysregulation and crosstalk of cellular signaling pathway in colon carcinogenesis. Crit Rev Oncol Hematol. 2013;86:251-77.
45. Zhang K, Civan J, Mukherjee S, Patel F, Yang H. Genetic variations in colorectal cancer risk and clinical outcome. World J Gastroenterol. 2014;20:4167-77.
46. Hsu HC, Liu YS, Tseng KC, Tan BC, Chen SJ, Chem HC. LGR5 regulates survival trough mitochondria-mediated apoptosis and by targetting the Wnt/β-catenin signaling pathway in colorectal cancer cells. Cell Signal. 2014;26:2333-42.
47. He S, Zhou H, Zhu X, et al. Expression of Lgr5, a marker of intestinal stem cells, in colorectal cancer and its clinicopathological significance. Biomed Pharmacother. 2014;68:507-13.
48. Todaro M, Gaggianesi M, Catalano V, et al. CD44v6 is a marker of constitutive and reprogrammed cancer stem cells driving colon cancer metastasis. Cell Stem cell. 2014;14:342-56.
49. Sikandar SS, Pate KT, Anderson S, et al. NOTCH signaling is required for formation and self-renewal of tumor-initiating cells and for repression of secretory cell differentiation in colon cancer. Cancer Res. 2010;70:1469-78.
50. Van Es JH, van Gijn ME, Riccio O, et al. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenoma into goblet cells. Nature. 2005;345:959-63.
51. Whissell G, Montagni E, Martinelli P, et al. The transcription factor GATA6 enables self-renewal of colon adenoma stem cells by repressing BMP gene expression. Nat Cell Biol. 2014;16:695-707.
52. Lombardo Y, Scopelliti A, Cammareri P, et al. Bone morphogeneic protein 4 induces differentiation of colorectal cancer stem cells and increases their response to chemotherapy in mice. Gastroenterol. 2011;140:297-309.
53. Ricci-Vittiani L, Mollinari C, di Martino S, et al. Thymosin beta 4 targeting impairs tumotigenic activity of colon cancer sem cells. FASEB J. 2010;24:4291-301.